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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/594,695	01/10/2007	Tetsu Akiyama	3190-101	8066

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KILYK & BOWERSOX, P.L.L.C.
400 HOLIDAY COURT
SUITE 102
WARRENTON, VA 20186

EXAMINER	
LI, QIAN JANICE	

ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/594,695	AKIYAMA ET AL.	
	Examiner	Art Unit	
	Q. JANICE LI	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-33* is/are pending in the application.

4a) Of the above claim(s) *1-12,16-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13-15 and 33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 September 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group III, claims 13-15 and 33, and the request for rejoining groups VI-V are acknowledged.

Upon search and consideration, the request has been granted in view of persuasive argument.

Claims 1-33 are pending, however, claims 1-12, 16-32 are withdrawn from further consideration by the Examiner, pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions, there being no allowable generic or linking claim. Claims 13-15, and 33 are under current examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 13-15 and 33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for providing heterozygous *Dlg* knockout mice, does not reasonably provide enablement for providing a genus heterozygous *dlg* gene knockout non-human mammals, and it does not reasonably provide enablement for using such mice for identifying a compound. The specification does not enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The factors to be considered when determining whether the disclosure satisfies the enablement requirements and whether undue experimentation would be required to make and use the claimed invention are summarized in *In re Wands*, (858 F2d 731, 737, 8 USPQ 2d 1400, 1404, (Fed Cir.1988)). These factors include but are not limited to the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, the breadth of the claims, and amount of direction provided. The factors most relevant to this rejection are the scope of the claims relative to the state of the art and the levels of the skilled in the art, and whether sufficient amount of direction or guidance are provided in the specification to enable one of skill in the art to practice the claimed invention.

1. The heterozygous dlg knockout non-human mammal

The claims embrace a genus of knockout non-human mammal (beyond mouse) deficient in one of the *Dlg* alleles. The specification discloses dlg heterozyougs (+/-) and homozygous -/- knockout mice made by homologous recombination using embryonic stem (ES) cells and targeting vectors (e.g. Example 1). With respect to ES cells, the state of the art is such that ES cell technology is generally limited to the mouse system at present, and only "putative" ES (ES-like) cells exist for other species (see *Moreadith et al.*, **J. Mol. Med.** 1997;75(3):208-16, e.g. S*ummary*). Note that "putative" ES cells lack a demonstration of giving rise to germline tissue (germline transmission) or the

whole animal (totipotency), a demonstration which is an art-recognized property of ES cells. Such a demonstration has not been provided by the specification or the prior or post-filing art with regard to the generation of any species of ES cells, other than the mouse. Accordingly, the claims appear to be only enabled for mouse. *Mullin et al.* supports this observation as they discuss the generation of non-mouse transgenics, *Mullins et al.* (**Journal of Clinical Investigation**, 1996) report that "ALTHOUGH TO DATE CHIMERIC ANIMALS HAVE BEEN GENERATED FROM SEVERAL SPECIES INCLUDING THE PIG, IN NO SPECIES OTHER THAN THE MOUSE HAS GERMLINE TRANSMISSION OF AN ES CELL BEEN SUCCESSFULLY DEMONSTRATED. THIS REMAIN A MAJOR GOAL FOR THE FUTURE AND MAY WELL REQUIRE THE USE OF NOVEL STRATEGIES WHICH DEPART WIDELY FROM THE TRADITIONAL METHODS USED IN THE MOUSE" (page 1558, column 2, first paragraph). Moreover, although the specification teaches methods to generate transgenic mice whose genome lacks one or both alleles of the *dlg* gene, the specification fails to teach methods of generating any other transgenic animals. It was known in the art, just murine subgenus of animal genus encompasses more than 1383 species of rodents, and one of skill would not be able to rely on the state of the transgenic art for an attempt to produce transgenic animals for the breadth claimed.

Without homologous recombination in ES cells, the animal has to be made through microinjection of fertilized eggs, and somatic cell nuclear transfer. It was highly unpredictable whether one could make the genus of dlg+/- animals, wherein the animal survive to maturity and develops tumor. This is because the aforementioned techniques were still under-development, and highly

unpredictable. The process of pronuclear microinjection is highly inefficient at the time of the filing. This is because the art of transgenic animals has for many years stated that the unpredictability lies with the site or sites of integration of the transgene into the target genome, which would vary among different species of animals. The elements of the particular construct used to make transgenic animals are held to be critical, and that they must be designed case by case without general rules. *Mullins* (**J Clin Invest**, 1996;97:1557-60) teaches, the major problems regarding pronuclear microinjection is that the exogenous DNA integrates randomly into chromosomal DNA, and that mouse-derived agents do not adequately prevent differentiation of stem cells in species other than mouse (left column, page 1558). *Mullins* concludes that "THE USE OF NONMURINE SPECIES FOR TRANSGENESIS WILL CONTINUE TO REFLECT THE SUITABILITY OF A PARTICULAR SPECIES FOR THE SPECIFIC QUESTIONS BEING ADDRESSED, BEARING IN MIND THAT A GIVEN CONSTRUCT MAY REACT VERY DIFFERENTLY FROM ONE SPECIES TO ANOTHER." (page S39, Summary).

The same is true for somatic cells nuclear cell transfer cloning. For example, *Denning* (Nat Biotech 2001;19:559-562) teaches difficulties of somatic cell cloning, "A SUBSTANTIAL NUMBER OF COLONIES WITH ONLY TARGETED CELLS SENESCED BEFORE THEY COULD BE PREPARED FOR NUCLEAR TRANSFER. THE HIGH ATTRITION RATE OF TARGETED CLONAL POPULATIONS SUITABLE FOR NUCLEAR TRANSFER REPRESENTS ONE OF THE MAJOR HURDLES OF GENE TARGETING IN PRIMARY SOMATIC CELLS" (left column, page 560). The unpredictability also lies with the faulty epigenetic reprogramming in nuclei cloning. Since the applicants have not disclosed other

animal species encompassed by the claims, it is highly unpredictable of the outcome of making any non-human mammal and their phenotypes. *Yanagimachi* (Mol Cell Endocrinol 2002;187:241-8) teaches, at a post-filing date, that "CLONING EFFICIENCY-AS DETERMINED BY THE PROPORTION OF LIVE OFFSPRING DEVELOPED FROM ALL OOCYTES THAT RECEIVED DONOR CELL NUCLEI-IS LOW REGARDLESS OF THE CELL TYPE (INCLUDING, EMBRYONIC STEM CELLS) AND ANIMAL SPECIES USED. IN ALL ANIMALS EXCEPT OF JAPANESE BLACK BEEF CATTLE, THE VAST MAJORITY OF CLONED EMBRYOS PERISH BEFORE REACHING FULL TERM" (Abstract), and "THUS FAR, CLONED OFFSPRING THAT SURVIVED BIRTH AND REACHED ADULTHOOD WERE THE EXCEPTION RATHER THAN THE RULE (page 243, left column, emphasis added). *Yanagimachi* goes on to teach, "THIS LOW EFFICIENCY OF CLONING SEEMS TO BE DUE LARGELY TO FAULTY EPIGENETIC REPROGRAMMING OF DONOR CELL NUCLEI AFTER TRANSFER INTO RECIPIENT OOCYTES. CLONED EMBRYOS WITH MAJOR EPIGENETIC ERRORS DIE BEFORE OR SOON AFTER IMPLANTATION" (abstract). *Wilmut* (Cloning Stem Cell 2003;5:99-100) teaches, "BY THE TIME OF DOLLY'S DEATH IN 2003, CLONES HAD BEEN DERIVED FROM ADULT CELLS OF SEVERN MAMMALIAN SPECIES, BUT THE SAME TECHNIQUES WERE NOT SUCCESSFUL IN SEVEN OTHERS, DESPITE INTENSIVE EFFORTS BY EXPERIENCED RESEARCH TEAMS. THESE INCLUDE RHESUS MONKEY, RAT, DOG, AND HORSE. THIS FAILURE EMPHASIZES THE IMPORTANCE OF DIFFERENCES BETWEEN SPECIES. THE DIFFERENCE MIGHT BE IN THE MOLECULAR MECHANISMS THAT REGULATE EARLY DEVELOPMENT OR IN ENABLING TECHNIQUES FOR OOCYTE RECOVERY, EMBRYO CULTURE, OR EMBRYO TRANSFER. SUCH DIFFERENCES HAVE ALREADY BEEN IDENTIFIED BETWEEN THE SPECIES FROM WHICH CLONES HAVE BEEN DERIVED", and "THE MOST STRIKING THING ABOUT THE TECHNIQUES THAT EMERGED DURING DOLLY'S LIFE IS THAT MAMMALIAN CLONING REMAINS A REPEATABLE, BUT INEFFICIENT

PROCEDURE…AN EXTRAORDINARY VARIETY OF ABNORMALITIES HAVE BEEN DESCRIBED IN CLONED EMBRYOS, FETURESES, AND OFFSPRING".

Polejaeva et al (Nature 2000;407:86) teach, "TO DATE, THE EFFICIENCY OF SOMATIC CELL NUCLEAR TRANSFER, WHEN MEASURED AS DEVELOPMENT TO TERM AS A PROPORTION OF OOCYTES USED, HAS BEEN VERY LOW (1-2%). A VARIETY OF FACTORS PROBABLY CONTRIBUTE TO THIS INEFFICIENCY THESE INCLUDE LABORATORY TO LABORATORY VARIATION, OOCYTE SOURCE AND QUALITY, METHODS OF EMBRYO CULTURE, DONOR CELL TYPE, POSSIBLE LOSS OF SOMATIC IMPRINTING IN THE NUCLEI OF THE RECONSTRUCTED EMBRYO, FAILURE TO REPROGRAM THE TRANSPLANTED NUCLEUS ADEQUATELY, AND FINALLY, THE FAILURE OF ARTIFICIAL METHODS OF ACTIVATION TO EMULATE REPRODUCIBLY THOSE CRUCIAL MEMBRANE-MEDIATED EVENTS THAT ACCOMPANY FERTILIZATION" (1st paragraph). Apparently, it was not, and has yet to become routine in the art to obtain a nonhuman transgenic/knockout livestock such as a *dlg* null pig having a tumorous phenotype to be useful for instantly claimed invention. The skilled in the art intending to practice the claimed invention would have to carry out undue experimentation to make the claimed non-human transgenic/knockout animals while the efficiency of the process would be expected low (<=1%) and phenotypic outcome of the animal is unpredictable due to the many variant factors as discussed *supra*.

Getting a desired phenotype in a transgenic animal is a major concern of transgenesis. The physiological art in general is known to be unpredictable (MPEP 2164.03), this is particularly true in the art of transgenic animals with respect to transgene behavior. Even within mouse species, targeting the same gene, the phenotype may be different. For example, *Levanon* (EMBO Reports

2003;4:560-4) teaches, "MANY KNOCKOUT (KO) MICE HAVE MULTIPLE, AND SOMETIMES COMPLEX, PHENOTYPIC DEFECTS. OFTEN, THE NULL PHENOTYPE DOES NOT SEEM TO RECAPITULATE THE KNOWN CELLULAR FUNCTION OF THE GENE. THIS IS FREQUENTLY THE CASE WHEN THE GENE BEING STUDIED HAS A DISTINCT TISSUE- AND/OR TEMPORAL-SPECIFIC FUNCTION THAT IS DIFFICULT TO REPLICATE IN CELL CULTURE. MANY HOMOZYGOUS KO MICE DIE *IN UTERO*, AND THOSE THAT ARE VIABLE OFTEN HAVE NUTRITIONAL OR IMMUNOLOGICAL DEFICIENCIES THAT CAUSE SECONDARY PHENOTYPES ASSOCIATED WITH ABERRANT GROWTH AND SURVIVAL." among two lines of Runx3 knockout mice, a stomach defect pertaining to gastric cancer was observed in one of the mutant strains, but not in the other (e.g. the abstract). *Levanon* points out the phenotype discrepancy is attributed to the genetic background of the mouse strains used, the differences in targeting constructs, and structural differences within the targeted genomic locus, and the promoter used, etc. (see entire article). Accordingly, it is highly unpredictable if the observed phenotype in the disclosed mouse could be reliably obtained in other strains of mice and other species of animals.

Accordingly, in view of the state of the art and the quantity of experimentation necessary for making a genus of *dlg* gene knockout animal, and having a skin tumor, the lack of direction or guidance provided by the specification as well as the absence of working examples with regard to any transgenic non-human animal whose genome comprises a heterozygous disruption of endogenous *dlg* gene and developing skin tumor, other than the exemplified *dlg* +/- and -/- null mouse, it would have required undue

experimentation for one skilled in the art to make and/or use the claimed invention.

Therefore, in view of the limited guidance, the lack of predictability of the art and the breadth of the claims, one skill in the art could not practice the invention without undue experimentation as it is broadly claimed.

2. The method for identifying a compound

The claims are directed a method of identifying a compound having an effect of enhancing the expression and/or function of either Dlg or sFRP or a compound that inhibits tumor formation in a Dlg +/- animal or cells from the animal. The claimed invention is based on the observation that Dlg+/- mice had reduced Dlg gene expression, develops skin cancer, and Dlg-/- mice have down-regulated sFRP gene

a). identifying a compound having an effect of enhancing the expression and/or function of Dlg.

The specification teaches that homozygous deficiency of Dlg gene died shortly after birth while heterozygous dlg+/- mice showed formation of skin tumor and NK cell lymphoma along with the growth (e.g. Specification, paragraph 0044-0045). The specification goes on to teach Dlg was not detected in the tumor formed but was detected in normal muscle cell within skin tissue, and concluded "*This finding suggests that the tumor formation in Dlg +/- mice may be due to the reduced expression and/or function of Dlg resulted from Dlg gene deficiency. Alternatively, it can be considered that natural mutation of Dlg gene may easily*

occur in Dlg +/- mice due to the deficiency in one of the alleles". As such, since the tumor formation was due to lack of *Dlg* gene in tumor cells, short of restoring the *Dlg* gene, a compound that enhance expression or function of *Dlg* would unlikely enhance the expression or function of a missing gene in tumor cells, and it is unpredictable whether any compound could enhance *Dlg* expression or function when one allele of the gene is missing. Accordingly, the claimed invention does not appear to be enabled in the absence of evidence to the contrary.

b). identifying a compound having an effect of enhancing the expression and/or function of sFRP.

Claims are also directed to a method of identifying a compound having an effect of enhancing the expression and/or function of sFRP. The specification teaches that Dlg -/- mice show reduced or missing gene expression of sFRP1 and sFRP2, respectively. However, the specification fails to teach the expression levels of sFRPs in *Dlg*+/- mice. It is unknown and unpredictable whether Dlg +/- mice also show reduced or missing gene expression of sFRP1 and sFRP2. The specification fails to shed light on this matter.

c*). identifying a compound that inhibits tumor formation.*

Based on the disclosure of the specification, the formation of tumor is associated with the deficiency of Dlg gene and the tumor formation was only present in the skin tissue. Logically, short of supplying *Dlg* gene, it is unlikely that any compound could inhibits tumor formation due to the deficient *Dlg* gene.

Therefore, in view of the disclosure of the specification, the lack of predictability of the art and the breadth of the claims, one skill in the art could not practice the invention without undue experimentation as it is broadly claimed.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Q. Janice Li** whose telephone number is **571-272-0730**. The examiner can normally be reached on 9:30 am - 7:30 p.m., Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Joseph Woitach** can be reached on **571-272-0739**. The **fax** numbers for the organization where this application or proceeding is assigned are **571-273-8300**.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

For all other customer support, please call the USPTO Call Center (UCC) at **800-786-9199**.

/Q. JANICE LI/
Primary Examiner, Art Unit 1633

Q. Janice Li, M.D.
Primary Examiner
Art Unit 1633

CJL
August 4, 2009